National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material 2389

Amino Acids in 0.1 mol/L Hydrochloric Acid

This Standard Reference Material (SRM) is a solution of 17 amino acids in a 0.1 mol/L aqueous solution of hydrochloric acid. Certified values are provided for all 17 amino acids. This SRM is intended primarily for the use in calibration of chromatographic instrumentation for the determination of the amino acids. A unit of SRM 2389 consists of five 2-mL ampoules each containing approximately 1.2 mL of the solution.

Certified Concentrations of Amino Acids

The certified concentrations and estimated uncertainties for the 17 amino acids are given in Table 1. These values are based on the results obtained from the NIST analytical determinations using liquid chromatography (LC) and a round robin study comprised of 13 participants conducted with the cooperation of the Association of Biomolecular Research Facilities (ABRF).

Table 1. Certified Concentrations of Amino Acids in SRM 2389

Amino Acid	Concentration mmol/L[a]
Alanine	2.51 ± 0.09
Arginine	2.94 ± 0.14
Aspartic Acid	2.50 ± 0.09
Cystine	1.16 ± 0.04
Glutamic Acid	2.47 ± 0.08
Glycine	2.45 ± 0.08
Histidine	2.83 ± 0.11
Isoleucine	2.39 ± 0.07
Leucine	2.48 ± 0.09
Lysine	2.47 ± 0.10
Methionine	2.43 ± 0.09
Phenylalanine	2.44 ± 0.08
Proline	2.44 ± 0.09
Serine	2.43 ± 0.09
Threonine	2.39 ± 0.08
Tyrosine	2.47 ± 0.09
Valine	2.44 ± 0.08

[a] The certified value is the equally-weighted mean of the NIST average and the round robin average. The expanded uncertainty reported above is two times the combined standard uncertainty for the certified value. The combined standard uncertainty for the certified value is one-half the sum, in quadrature, of the NIST and round robin standard uncertainties and conforms to NIST guidelines.[1]

Gaithersburg, MD 20899
December 6, 1993

Thomas E. Gills, Acting Chief
Standard Reference Materials Program

(over)

NOTICE AND WARNING TO USERS

Handling: This material contains 0.1 mol/L hydrochloric acid and should be handled with care because it is corrosive and may cause burns. Use proper disposal methods.

Expiration of Certification: The certified values are valid, within the limits specified, for three years from the date of shipment from NIST. In the event that the certification should become invalid before then, users will be notified by NIST. Please return the attached registration card to facilitate notification.

Storage: Sealed ampoules, as received, should be stored in the dark at approximately 4 °C.

Use: Sample aliquots should be drawn for analysis at 20-25 °C immediately after opening the ampoule. The ampoules were sealed under nitrogen and although the amino acids are relatively stable, some long term degradation may occur.

The coordination of the technical measurements leading to the certification were performed in the NIST Organic Analytical Research Division by S.A. Margolis and S.A. Wise.

Preparation and analytical determinations were performed in the NIST Organic Analytical Research Division by S.A. Margolis.

Statistical consultations on the experimental design and the evaluation of the data were provided by S.B. Schiller of the NIST Statistical Engineering Division.

The round robin study was conducted with the cooperation of the Association of Biomolecular Research Facilities (ABRF). All participants in the study were members of the ABRF and are listed in Appendix 2.

The technical and support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the Standard Reference Materials Program by J.C. Colbert.

Preparation and Analysis

NIST LC Measurements: All chemicals used in the preparation of this SRM were obtained from commercial sources. The amino acid solution was prepared by weighing the individual amino acids, concentrated HCl, and water and mixing until the amino acids were completely dissolved. The total mass of this solution was measured. The concentration of each amino acid was calculated using the density of 0.1 HCl at 23 °C of 0.9990 g/cm^3. The purity of each amino acid was examined by LC analysis for contamination with other amino acids. Arginine, glutamic acid, serine, and threonine contained from 0.2 to 3.5% amino acid impurities. The remaining amino acids contained less than 0.25% of amino acid impurities. The gravimetric values for each amino acid were adjusted to account for these impurities. The amino acids were not examined for other organic or inorganic impurities except for histidine, which was found to be a mixture of the free base and the chloride salt using nuclear magnetic resonance spectroscopy. The bulk solution was dispensed under dry nitrogen in 1.2 mL aliquots into 2-mL amber ampoules which were then flame sealed.

Aliquots from 15 randomly selected ampoules were analyzed in duplicate by LC on an Amino-Tag Column (Varian Analytical Instruments, San Fernando, CA) employing UV detection (at 260 nm) of the precolumn derivatized 9-fluorenylmethyl chloroformate (FMOC) derivative of the amino acids. Three external standards (phenylalanine, tyrosine, and histidine) were used for quantification purposes. The external standards were prepared gravimetrically in 0.1 mol/L KOH (phenylalanine and tyrosine) or water (histidine) at a concentration suitable for measuring their absorbance at 258.2, 298, and 211 nm, respectively.[2] The exact concentration was calculated for each external standard using its molecular absorbance at the indicated wavelength.[2] These solutions were further diluted by weight to prepare four mixtures of the three amino acids at four different concentrations bracketing the concentration of the amino acids in the SRM. These were used to determine the calibration curves. Extensive investigation of the FMOC derivative indicated that all FMOC amino acid derivatives except tyrosine, lysine, and histidine exhibit similar UV molecular absorbances. Similar UV molecular absorbances were also observed for tyrosine, lysine, and histidine which are doubly derivatized by FMOC.

Round Robin Analyses: The participating labortories analyzed two independent samples using the method(s) routinely used in their laboratory. These included a variety of instruments, standardization techniques, and methods. Three basic types of derivatizing agents were used by the laboratories: Ninhydrin (6 laboratories), phenyl isothiocyanate (6 laboratories) or fluorescamine (1 laboratory). Calibration was achieved by using either a commercial mixture of amino acids or an internal standard such as norleucine.

REFERENCES

[1] Taylor, B.N., and Kuyatt, C.E., NIST Tech. Note 1297, Jan. 1993.

[2] Handbook of Biochemistry, Proteins, G. Fasman, ed., vol. I, pp. 183-191, (1976) CRC Press, Cleveland, OH.

SUPPLEMENTAL INFORMATION

Noncertified Quantitative Values

Appendix 1 contains supplementary analytical results and information obtained during the certification of SRM 2389, including (a) the gravimetric value from the preparation of the solution, (b) the results of the NIST LC measurements, and (c) the results of a round robin study on this SRM. Appendix 2 contains a list of the participants in the round robin study.

Appendix 1. Summary of the Analytical Results for SRM 2389 [a]

Amino Acid	Amino Acid Concentration [b] (mmol/L) Gravimetric Value[c]	NIST LC Measurements[d]	Round Robin Study[e]
Alanine	2.50	2.62 ± 0.07	2.39 ± 0.05
Arginine	2.83	3.03 ± 0.11	2.85 ± 0.09
Aspartic Acid	2.55	2.57 ± 0.07	2.44 ± 0.06
Cystine	1.20	1.16 ± 0.06	1.17 ± 0.05
Glutamic Acid	2.44	2.53 ± 0.06	2.41 ± 0.05
Glycine	2.51	2.54 ± 0.07	2.37 ± 0.05
Histidine	2.49	2.77 ± 0.06	2.88 ± 0.09
Isoleucine	2.54	2.36 ± 0.05	2.41 ± 0.05
Leucine	2.60	2.56 ± 0.08	2.40 ± 0.05
Lysine	2.51	2.52 ± 0.08	2.42 ± 0.06
Methionine	2.53	2.48 ± 0.06	2.37 ± 0.06
Phenylalanine	2.58	2.44 ± 0.06	2.44 ± 0.05
Proline	2.50	2.50 ± 0.06	2.37 ± 0.06
Serine	2.47	2.50 ± 0.07	2.35 ± 0.05
Threonine	2.44	2.41 ± 0.06	2.38 ± 0.04
Tyrosine	2.49	2.52 ± 0.08	2.42 ± 0.05
Valine	2.55	2.48 ± 0.06	2.40 ± 0.05

[a] The summary of results given above is presented as supplemental information to the certified values. **The certified values, however, are the best estimates of true concentration for calibration, method validation, and other quality control purposes.**

[b] The amino acid content is based on the molecular weight of the free amino acid except for histidine which is based on the molecular weight of the HCl salt. The histidine is actually a mixture of the free base and the salt which accounts for the discrepancy between the gravimetric and assayed values.

[c] The gravimetric value is based on the weighed amount of each amino acid used to prepare the solution.

[d] This is the mean value for duplicate measurements on 15 discreet samples (n = 30) and the standard uncertainty. To determine the standard uncertainty, a 95% confidence interval for the average corrected LC peak area was intersected with 95% confidence bands for the calibration curve. The confidence interval for the average corrected peak area includes between-day as well as within-day variance components for the LC measurements. The standard uncertainty is the 95% confidence interval for the mean, divided by 2.

[e] The round robin average is the average of the lab means and the standard uncertainty is the standard deviation of that average. n = 26 independent samples except cystine (n = 22) and proline and valine (n = 24).

Appendix 2. Laboratories Participating in the Round Robin Study

Stanford University Medical Center	Palo Alto, CA
Pfizer Central Research	Groton, CT
Abbott Laboratories	Abbott Park, IL
Biogen Corporation	Cambridge, MA
Massachusetts Institute of Technology	Cambridge, MA
Ciba-Geigy Biotech	Research Triangle Park, NC
Hoffman-LaRoche Inc.	Nutley, NJ
W. Alton Jones Cell Science Laboratory	Lake Placid, NY
Rockefeller University	New York City, NY
Wistar Institute	Philadelphia, PA
AAA Laboratory	Mercer Island, WA
Medical College of Wisconsin	Milwaukee, WI
Beckman Company	Palo Alto, CA